critical regulators of cytoskeletal remodeling, cell adhesion and motility. γ-synuclein alters binding between Rac1 and PLCβ2. This observations and activity measurements suggests that γ-synuclein mediates cell motility and invasiveness through Rac1-PLCβ2 pathway.

3595-Pos

PTEN and Ci-VSP Show Similar Phosphoinositide Binding Preferences

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The *Ciona intestinalis* voltage sensor containing phosphatase (Ci-VSP) is a voltage dependent phosphatidylinositol phosphatase with two main domains, the voltage sensing domain (VSD) and the phosphatase domain (PD). Ci-VSP's PD bears homology with the phosphatase domain of the tumor suppressor protein PTEN (phosphatase and tensin homolog deleted chromosome 10). Recently, it has been proposed that the linker between the VSD and PD Ci-VSP constitute a Phosphoinositide Binding Motif (PBM). The PBM of Ci-VSP shares high homology with PTEN's N-terminal, which, in turn, is known to bind $PI(4,5)P_2$ leading to an allosteric activation of PTEN. Similarly to the PBM of PTEN, we have found that a peptide representing the Ci-VSP's PBM ($Ci\text{-}VSP_{240\text{-}271}$) binds $PI(4,5)P_2$ significantly more strongly than other phosphatidyinositol bisphosphates. A Ci-VSP chimera created by replacing Arg 257 and Lys 258 with the amino acids found at the corresponding positions in the PTEN sequence, Gln 16 and Glu17, lead to a peptide that showed significantly reduced binding to $PI(4,5)P_2$. While $Ci\text{-}VSP_{240\text{-}271}$ exhibited a mixture of random and a-helical secondary structural elements, it was found that the chimeric Ci-VSP showed an increased b-sheet content. Molecular dynamics simulations were performed using the package NAMD and showed that the peptide does not form a helical structure and its charged residues interact in a pairwise fashion with $PI(4,5)P_2$. Those pairs are form by R245 and R246, K252 and R253, and R254 and R257. Experimentally, the binding of the PBM has a great influence on the rate of return of the sensing charges. However, in a deletion mutant lacking the PD we observed that the PBM alone is not enough to limit the rate of return of the sensing currents. These observations strongly suggest that the binding of the PBM might be stabilized by the PD.

3596-Pos

Membrane Phosphatidylserine and Plasma Ca2+ Levels Switch Factor Xa from an Inactive Dimer to an Active Monomer

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The main role of factor Xa(fXa) in the coagulation cascade is association with factor Va to catalyze the proteolytic activation of prothrombin to thrombin. Phosphatidylserine (PS) triggers both this fXa-fVa association and formation of inactive fXa dimers both in solution and on a membrane. We show here that binding of fXa to PS-containing membranes promotes proteolytic activity at low Ca2+ but inhibits it at high Ca2+ concentration, with the transition of FXa from active to inactive form on PS-containing membranes being a sigmoidal function of Ca2+ concentration. We modeled this membrane regulation of fXa activity to obtain kcat/KMdimer = 0 M-1s-1 and Kd,surfacedimer = (40 ± 25)●10-15 mol/(dm)2 at 4 mM Ca2+. This surface dimerization constant corresponds to a solution-phase Kddimer = 1 nM at 10 μM lipid concentration, nearly the same as observed (20 nM) for short-chain PS-triggered formation of fXa dimers in solution. fXa was activated by membrane binding below 1.1 mM Ca2+ but inactivated above this Ca2+ concentration. This resulted because the dimerization constant increased with decreasing Ca2+ concentrations (calculated at at 3.08, 1.54, and 1.16 mM Ca2+, respectively). Just below the normal range of free plasma Ca2+ concentration (1 - 1.3 mM), addition of PS-containing membranes promotes factor Xa activity, while at physiological [Ca2+], fXa is inhibited by dimerization, which depends critically on [Ca2+]. Supported by USPHS grant HL072827.

3597-Pos

Quantitative Analysis of Binding Affinities of PI(4,5)P2 Sensor Domains in Living Cells by Using the Voltage-Controlled PI(4,5)P2-5'-Phosphatase, Ci-VSP

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Dynamic changes in the phosphoinositide (PI) concentration in the cell membrane play an important role in the regulation of many cellular processes. Of particular interest is the PI phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$), a genuine signaling molecule that, e.g., controls the function of some ion channels.

Plasma membrane PI concentration in living cells is often monitored with genetically encoded fluorescence labeled PI-binding probes such as the $PI(4,5)P_2$ specific $PLC\delta_1$-PH-GFP and tubby-GFP probes. Knowledge of their $PI(4,5)P_2$ affinities is essential in selecting the most suitable probes and for appropriate interpretation of experimental results. These affinities of $PI(4,5)P_2$ probes apparently differ and can be modified by mutagenesis; however, a quantitative analysis of affinity in vivo is lacking.

To address this issue we employed the voltage dependent $PI(4,5)P_2$-5′-phosphatase Ci-VSP which alters plasma membrane $[PI(4,5)P_2]$ as a function of membrane voltage in a graded and reversible manner (Halaszovich et al., 2009, JBC 284:2106-13). We co-expressed Ci-VSP with various GFP-tagged $PI(4,5)P_2$ probes and used total internal reflection microscopy (TIRF-M) to measure membrane association of the probe at different membrane voltages. Generally, the TIRF signal decreased upon depolarization, indicating the translocation of the probe from the cell membrane into the cytosol in response to the decrease in $[PI(4,5)P_2]$. Voltage-dependent changes of TIRF signals allowed construction of fluorescence-voltage relations for each probe that can be used as surrogate $PI(4,5)P_2$ binding curves. Comparison of these curves defined the rank order of $PI(4,5)P_2$ affinities of the various probes.

We conclude that Ci-VSP is a valuable tool for analyzing $PI(4,5)P_2$ affinities in living cells, which we demonstrated by determining the affinities of fluorescence labeled $PI(4,5)P_2$-probes.

Supported by DFG grant OL 240/2 and SFB 593 to D.O.

3598-Pos

Optical Control of Cell Death: Translation of a Temporal Process to a Spatial Display

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The ability to arrest a dynamic physiological process and display the progression as a sequence of events has proved successful for the imaging of transport through transmembrane channels under cryogenic conditions. If cellular processes that advance in response to insult could be similarly arrested and displayed, it would open the door to investigation of biophysical and biochemical questions that are currently difficult to address. We have developed such a spatial cellular array, which might be utilized to study a broad spectrum of stress-induced physiological processes. As an illustration, we show that the process of optically induced cellular apoptosis can be translated from the temporal progression to a spatial array. Following graded doses of short-wavelength ultraviolet radiation, cells presented to a surface undergo progressively more advanced stages of the apoptotic cascade, depending upon their position in the array, as measured by caspase-8 and caspase-3 activation. This broadly applicable tool, exemplified here by the display of optically induced apoptosis, could facilitate the study of a wide range of optically and oxidatively stiumulated processes.

3599-Pos

Patch Clamped Giant Unilamellar Vesicles Containing Reconstituted Ion Channels

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There is increasing interest in how the chemical and physical properties of lipid membranes affect the function of mammalian ion channels. As evidenced by recent work on KvAP and Kv1.2, as well as studies of the physical nature of patches themselves, these effects are both intriguing and difficult to study. For the TRP family of ion channels these questions are especially important as membrane lipids, such as phosphoinositide (4,5) bisphosphate (PIP2), play an active role in regulating their functional properties. Although much work has been done to address the mechanism by which lipids regulate TRP channels in intact cells and excised patches, the physical interactions that govern channel regulation are still unknown. We report here on our efforts to establish high resolution control over membrane chemical composition and physical properties. We find that giant unilammelar vesicles can be synthesized with desired lipid compositions and subsequently studied using patch-clamp techniques. Reconstitution of functional TRP channels into these synthetic lipid membranes would provide a well-controlled experimental paradigm for studying the function and mechanism of channel-lipid interactions.